EFFECTS OF PROPRANOLOL, OXPRENOLOL AND ATENOLOL ON RAT BEHAVIOUR WITHIN THE OPEN FIELD AND EMERGENCE TEST

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ABSTRACT

Open field and emergence test activity was observed in three separate experiments using a total of 144 male and female albino rats. Subjects were injected with either propranolol (Experiment One), oxprenolol (Experiment Two), or atenolol (Experiment Three) in doses of 5, 10 or 20mg/kg approximately 30 minutes prior to testing. The aim was to determine whether the drugs exerted behavioural effects on free responding, and whether such influences could be interpreted in terms of an anxiolytic action. Behavioural variables measured were walking, rearing, grooming, centering, cornering, transitions, half body emergence time and total body emergence time. Faecal boli were also counted at the end of each subject's observation time in the open field apparatus.

Analyses of Variance revealed significant influences of propranolol on rearing, walking and cornering behaviour. Oxprenolol significantly affected rearing, transitions and emergence measures. Atenolol significantly affected rearing and half body emergence. It was concluded that, within the interpretative limitations of the behavioural measures used, an anxiolytic action could be postulated for propranolol administered to male rats and possibly oxprenolol also.

CHAPTER ONE

INTRODUCTION

Beta-adrenergic receptors in the sympathetic nervous system

The sympathetic nervous system, as part of our autonomic nervous system, mediates stress-induced energy mobilization within the body. Sympathetic functions prepare the body for the increased level of motor activity involved in attack, defense or escape reactions to stress. Ahlquist (1948) noted that sympathetic nervous system receptors may be classified as α or β according to the response of various tissues to sympathomimetic amines. Beta receptors can be further divided into β_1 (cardiac) or β_2 (vascular, bronchial and gastrointestinal smooth muscle) receptors. Alpha receptor stimulation leads to smooth muscle contraction in blood vessels, the uterus and iris (Gibson, 1974). Stimulation of β receptors leads to smooth muscle relaxation in the bronchi, intestine and uterus, along with an increase in the rate and force of cardiac contraction.

Three known candidates for β receptor agonists within the sympathetic nervous system exist. These are dopamine, nor adrenaline and adrenaline. Adrenaline has both α and β-agonist effects and, although occurring in only small amounts in the brain, is the most potent physiological β receptor agonist known. Nor adrenaline is primarily an α receptor agonist although it does serve as a β receptor agonist in the heart, for example. Another receptor for dopamine exists in the brain and dopamine makes a very poor β receptor agonist in the peripheral nervous system (Carlsson, 1975).

Of interest in this study is the effect of three drugs which block β adreneregic receptors on some aspects of rat behaviour.

The sympathetic nervous system and anxiety

Both psychological and somatic symptoms occur when anxiety is experienced. For example, symptoms of tension, fear, difficulty in concentrating or apprehension may occur in combination with tachycardia, palpitations, tremour or gastrointestinal upset. Physiological changes include an increase in pulse rate, systolic blood pressure, muscle activity and respiratory rate, a decrease in salivation and finger pulse volume, perspiration (diaphoresis) and excessive pupil dilation (Hayes and Schulz, 1983). These changes result mainly from β-sympathetic over-activity. Such overactivity may form a chemical base to the suggestion made by James (1890) that somatic symptoms can reinforce anxiety (Carruthers, 1975). The James-Lange hypothesis suggests that the experience of anxiety is secondary to the perception of bodily changes. That is, through a positive feedback loop there is cerebral recognition of somatic symptoms of sympathetic activity and their association with previous experiences of anxiety (James, 1984).

There is a class of drugs, known as β blockers, which inhibit β adrenergic activity. As such they disrupt any indications of emotionality mediated by β receptors. This had led to their application to the treatment of anxiety in a clinical setting. Their usefulness in this area remains unclear. Benzodiazepines are currently the drugs of choice in the treatment of anxiety. These appear to be effective in decreasing both psychological and somatic symptoms. Since anxiety disorders tend to be episodic, optimal response to benzodiazepines occurs when therapy is initiated during exacerbation periods and administration is restricted to brief intervals of 2-3 weeks. There is little evidence to suggest that

such drugs continue to be effective for longer periods of time (Hayes and Schulz, 1983). High doses administered for some time are also associated with the risk of dependence.

An alternative to the benzodiazepines is the barbiturate class of drugs. Their use also carries with it the risk of dependence.

Because of these adverse consequences there is current interest in other methods of anxiety treatment. One such alternative may the be β blockers.

β blocker uses

Beta blocking drugs were initially used to treat cardiac complaints associated with sympathetic overactivity. Today such uses include the treatment of sinus tachycardia, cardiac arrhythmias, obstructive cardiomyopathy and angina pectoris.

Granville-Grossman and Turner (1966) were the first researchers to investigate the effects of β blockers on anxiety. Only autonomically mediated symptoms such as palpitations, sweating and diarrhoea were significantly affected. Physical symptoms, non autonomically mediated, such as headache, were not affected. Neither were mental symptoms of worry, tension and fear. Beta blocker action thus appears to be selective. While a series of studies have shown the drugs to be useful in treating clinical anxiety their effectiveness seems to be limited in the treatment of anxiety induced in normal subjects. Lader (1977) suggested why this might be. The bodily symptoms of anxiety in normal subjects are mild and explicable. Any reduction due to β blockade would be unimportant. Compounds which influence the central, cognitive aspects should be more effective. Beta blockers should be of help to normal subjects experiencing the irrational feelings associated with phobic stimuli and the heightened anxiety experienced before examinations, in

stage fright and public speaking. They should also be effective in treating clinically anxious patients with somatic symptoms. In both these groups peripherally mediated, irrational somatic symptoms are dominant. In psychically anxious patients, where many of the physiological changes are not disturbing and subjective symptoms are dominant, drugs which affect the cognitive aspects of anxiety directly should be more effective.

Various other uses for the β-blockers have been suggested. Animal experiments have shown that the β blocker propranolol inhibits brain binding of morphine in mice (De Feudis and Grosz, 1972) and modifies some behavioural changes induced by morphine in rats (Block and Grosz, 1974). From these have come the suggestion that the drug could be of use in the treatment of opioid dependence. Findings with respect to humans have largely been negative however.

A use for β blockers has been implicated in the treatment of Parkinsonism, essential tremour and lithium-induced tremour. Beta blockers decrease the adrenaline-induced increase in Parkinsonian tremour amplitude. Overall, reports on the effectiveness of the drugs in treating this particular disorder are inconsistent (Agnoli, Cerone, Ruggieri, Cappenberg and Aloisi, 1977), as are those associated with lithium induced tremour (Tyrer, 1981).

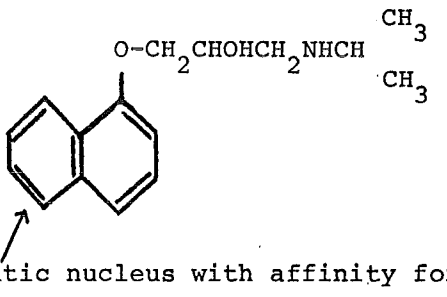
There have been some reports of successful treatment of psychoses using β blockers. For example, Atsmon (1972) reported that 600mg of propranolol per day removed the abdominal symptoms and psychosis of a woman diagnosed as suffering from porphyria associated with a symptomatic psychosis. The symptoms reappeared upon withdrawal of the drug while readministration removed them again.

β blocker chemical structure

The β blockers are characterised by a catecholamine-like chemical structure. It is this which allows for reversible blocking of β-adrenergic receptors in the sympathetic nervous system.

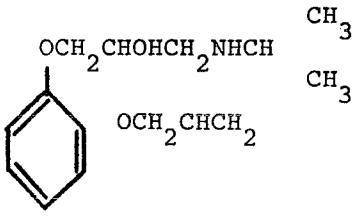
The drugs used in the present study were propranolol (®Inderal), oxprenolol (®Trasicor) and atenolol (®Tenormin).

Propranolol

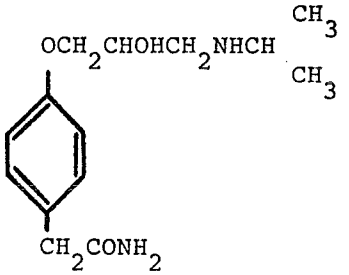


aromatic nucleus with affinity for β receptors

Oxprenolol



Atenolol

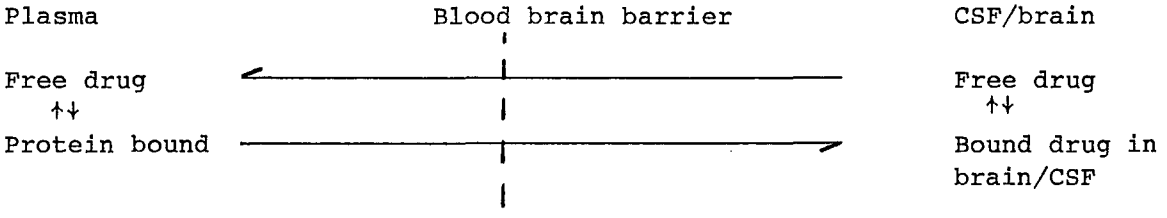


Properties of the β blockers

All β blockers used clinically are known to block β_1 receptors and most therapeutic effects are based on this blockade. Non selective β blockers block β_2 adrenoceptors as well. Only in the treatment of glaucoma and certain forms of tremour is β_2 blockade known to be the basis of the therapeutic effect (van Zwieten and Timmermans, 1983). Atenolol is a cardioselective β blocker, it does not show central β blocking activity. Both propranolol and oxprenolol are non selective.

Propranolol and oxprenolol, when injected intracerebrally or intracerebroventricularly, decrease cell membrane permeability for ions such as sodium, potassium and calcium. This membrane stabilizing or local anesthetic effect is not exhibited by atenolol.

Some β blockers, as well as preventing the binding of catecholamines to adrenergic receptors, cause mild stimulation of the receptors. Such intrinsic sympathomimetic activity is shown by oxprenolol and appears to be dose dependent (Saxena and Forsyth, 1976). The β blocking drugs differ in their ability to cross the blood-brain barrier, depending on the plasma concentration of the free drug and its lipophilic structure. Lipophilic agents such as propranolol pass easily into the central nervous system. Oxprenolol, being less lipophilic, crosses less readily and atenolol, which is hydrophilic, crosses only poorly. The theoretical distribution of a blocker in the plasma and central nervous system follows the pattern



(Cruikshank, Neil-Dwyer, Bartlett & McAinsh 1982)

Distribution of lipophilic β blockers within the blood/cerebrospinal fluid/brain compartments occurs within minutes. Equilibration of atenolol is considerably slower (Cruikshank et al., 1982). Finally, the different β blockers vary in the way they are metabolised. Lipophilic compounds (eg. propranolol, oxprenolol) are extensively metabolised in the liver. Hydrophilic compounds are excreted largely unchanged by the kidneys (Johnsson and Regardh, 1976). Most β blockers have a short half life, for example atenolol's plasma half life is 6-9 hours after a single dose while propranolol's is 2-3 hours (Kelly, 1978).

A variety of effects of β blockers in the periphery and central nervous system have been noted. Explanation of these characteristics is made difficult by the varying properties of the different drugs. This is seen at present in the sometimes inconclusive or contradictory findings in antianxiety research.

β blocker anxiolytic mechanisms of action

Three possible ways in which the β blockers could exert an anti-anxiety effect have been suggested.

1. β blockers act directly and specifically on CNS β adrenergic receptors

β adrenergic receptors have been identified in the CNS (eg. Davis and Lefkowitz, 1976; Margules, 1971). The CNS is known to contain extensive adrenergic pathways linking different brain areas, including those involved in fight/flight functions. The locus coeruleus, for example, found in the brain stem, contains many adrenergic neurons and projects widely to the cerebral cortex. It is thought to play a key role in the genesis of anxiety (Lader, 1974).

Evidence for a direct (although not necessarily specific) mode of action comes from the observation that some of the central effects seen

after systemic β blocker administration are mimiced after direct application to or into the brain.

For those drugs which readily cross the blood brain barrier a direct specific central mechanism of action cannot be ruled out. Drugs which penetrate the CNS to a lesser degree may act in other ways, unless given in large doses.

2. β blockers act directly on the CNS independently of a specific adrenergic receptor

It may be that the antianxiety effects seen after administration are due to properties of the drugs other than β receptor blockade. For example, Speiser and Weinstock (1973) demonstrated that both dl-(0.2 and 0.5 mg/kg) and d-(0.5-1.0 mg/kg) propranolol produce sedation in isolation-induced hyperactive mice. The d-isomer lacks significant β-blocking properties. The sedative effect observed may be attributable to another property of the drug, such as its central cell membrane stabilizing effect, known to occur in both isomers. Bainbridge and Greenwood (1971) found similar results with the propranolol isomers and their tranquillizing effect in rats conditioned to expect electric shock or made hyper-reactive through septal lesions. Whether these animal studies can be related to the mechanism of drug action in humans is questionable since the doses used were much higher than those in therapeutic practice. There is some evidence for such a mechanism operating in human subjects eg. Farhoumand, Harrison, Pare, Turner and Wynn (1979), with respect to oxprenolol.

3. β blockers act on peripheral receptor sites. Neural and/or homoral feedback results in the central awareness of anxiety reduction

Estler and Ammon (1969) found no central effect of the (hydrophilic) β blocker practolol in man. Bonn and Turner (1971) showed however that practolol is able to relieve anxiety in human subjects.

Information about peripheral effects of the drug must have reached the CNS in such a case. There is evidence for this type of feedback mechanism. For example, it is known that a reduction in heart rate decreases, indirectly, subjective anxiety. Also, the activity of some CNS structures can be changed by objective changes in neuro-humoral feedback due to altered levels of peripheral autonomic activity. For instance, changes in respiratory rate and volume lead to slight O_2 and CO_2 pressure variations, which in turn alter levels of central activity. Some areas of the brain are located outside the blood-brain barrier. These include the area postrema, subfornical organ and the subcommisural organ. Any β blocking action on these structures is, by definition, peripheral.

It seems that a single mechanism of β blocker action is not exclusively responsible for the ultimate antianxiety effects observed. Which mechanism is operational depends on the species, behaviour model being studied, the drug employed and the initial level of activity of the particular function being studied (Koella, 1977). With respect to the anxiolytic properties of blockers the evidence, although not unequivocal, favours a peripheral mechanism in human subjects. In animal studies anxiety can be measured only indirectly. From these studies there is majority support, although once again not unequivocal, for a nonspecific central action.

Anxiolytic effects of the β-blockers: Animal studies

Experiments on animals have traditionally introduced stressors into the environment in order to induce an emotion paralleling human anxiety. Such stressors include prolonged isolation, conflict tasks, drug and surgical manipulations. It is unclear, however, whether or not stress-induced states in animals relate to anxiety in humans. Lader (1974) likens the gross alterations in behaviour seen in the

"experimental neuroses" more to the acute traumatic neurotic syndrome (eg. following major disasters such as earthquakes) than to anxiety states. Thus, more subtle animal models must be used. In the experiments described in this thesis exposure to a novel environment provides the source of anxiety.

Of the three drugs administered in this study propranolol has been investigated most extensively within the experimental neurosis paradigm. Some researchers have found an apparent antianxiety effect with this drug, others have found none. Robichaud, Sledge, Hefner and Goldberg (1973), using the experimentally induced conflict task of Geller and Seifter (1960) obtained what they described as a suppression of behaviour in male Long Evans hooded rats. The rats, maintained at 70% of their mature body weight, were able to bar press for food accompanied by electric shock. Propranolol (5mg/kg i.p.) had no effect on this suppression. It also had no effect on the avoidance performance of male albino mice (C.F.W. stain) partially trained to avoid electric shocks in the shuttle box. Both of these experimental models are predictive of clinical activity for several classes of antianxiety agents including the benzodiazepines. When administered in combination with chlordiazepoxide propranolol had no influence on the effect of the tranquillizer in either procedure. It may be that the dose of propranolol used in this experiment was not high enough to bring about a behavioural change, although Sepinwall, Grodsky, Sullivan and Cook (1973) found little effect of doses from 2.5 to 80mg/kg adminstered orally. These experimenters used a similar lever pressing conflict procedure on male Charles River albino rats. Typically with this procedure a dose related increase in punished responding is seen, frequently several 100% above control levels. In this investigation the 26% increase at a dose of 20mg/kg was the only one which reached statistical significance.

These results contrast with those of Bainbridge and Greenwood (1971) who reported an apparent tranquillizing effect of 10 and 20mg/kg p.o. propranolol on male albino rats (Alderly Park strain 1) conditioned to expect an electric shock. A decrease in the hyperirritability of septally lesioned rats was also seen after propranolol administration. The authors saw both conditions as plausible models of clinical anxiety. Rather than a general depression of behaviour the tranquillizing effect was interpeted as an antianxiety effect since dl-propranolol caused a significant increase in preening behaviour immediately following CS presentation. Propranolol had its effect in common with four representative central nervous system depressants also tested; chlorpromazine, haloperidol, chlordiazepoxide and phenobarbitone. D-propranolol had no such effect. A second series of experiments involving dl-propranolol and phenobarbitone showed that both drugs significantly decreased freezing and increased exploratory behaviour. These effects were interpreted as resulting from behavioural disinhibition and a decrease in tension/fear.

Weinstock and Speiser (1973) found that both d- and dl-propranolol (0.2-0.5mg/kg) decreased isolation-induced hyperactivity, sniffing and rearing in male albino rats (Wistar strain) in the open field. Practolol, another β blocker, was similarly effective. The doses used were without effect on control subjects housed 4-6 per cage. 20mg/kg dl-propranolol was necessary to reduce the activity of the group-housed rats. Parallel results have been found in humans. Propranolol has been shown to sedate psychotic patients but, even at relatively high doses, does not show any significant central effects on normal human subjects (Lader and Tyrer, 1972). Archer (1973), for example, interpreted decreased locomotor activity in terms of increased rather than decreased anxiety. This makes the interpretation of these results unclear. It can be concluded, therefore, that with respect to propranolol and experiments which attempt to manipulate animal anxiety levels findings are inconclusive and their validity has been questioned.

Hughes (1981) investigated the effect of oxprenolol (10 and 20mg/kg i.p.) on emergence latency, novelty preference, rearing and ambulation in an exploration box. Both doses decreased emergence latency - a possible antianxiety effect. Because there was no effect observed on centrally mediated novelty preference and rearing it was concluded that a peripheral anxiolytic effect was probably operational. Centrally acting diazepam was also tested. Rearing and novelty preference, as well as emergence latency, were decreased in a dose related manner. Subjects of this experiment were both male and female hooded rats (randomly derived strain).

Oxprenolol (100mg/kg p.o.) also has shown an antianxiety effect on the development of hyperthermia in a shock enclosure. Noble and Delini-Stula (1976) subjected male albino Wistar rats to inescapable shocks once a day for five days. Exploratory behaviour (head dipping in a hole board), suppressed by previous exposure to shocks, was uninfluenced by oxprenolol. Within the oxprenolol treated group grooming decreased from the first day of the trial onwards. Tinbergen (1952) saw grooming as a manifestation of fear while O'Kelly (1940) saw it as a non adjustive reaction to stress. Hyperthermia has been interpreted as a somatic manifestation of emotional disturbance (Delini-Stula and Morpurgo, 1970; Miller, 1969).

Thus the small amount of research involving oxprenolol does appear to support an antianxiety effect. Atenolol's role in animal anxiety remains largely uninvestigated directly. Weinstock and Weiss (1980) investigated the effects of β blockers including dl-propranolol, d-propranolol and atenolol on male mice (Sabra Hebrew University strain). Insofar as aggression may be one reaction to anxiety this investigation looked at the effect of the β blockers on anxiety. While dl-propranolol showed a significant antiaggressive effect in doses less than 5mg/kg, neither atenolol nor d-propranolol were active, even at doses up to 20mg/kg.

Measures of emotionality

How is it possible to measure anxiety in animal subjects? Behaviours which have been interpreted as being influenced by anxiety include freezing, rearing, grooming, active escape, ambulation, defecation and emergence. At their most specific these behaviours might be seen to reflect an underlying emotionality construct. To see this emotionality as anything more specific involves the danger of anthropomorphism. Labelling a behaviour as reflecting a particular emotion poses the question - how can the emotions be differentiated? Both behaviourally and physiologically there is little to discriminate between similar intensities of differing emotions.

Emotionality describes aspects of the generally excited and upset condition of animals faced with unfamiliar situations (Doyle and Yule, 1959). Measuring the relative emotionality of animal subjects typically involves the open field environment. In the open field an animal is faced with a large, empty, spatially bounded, illuminated area. Since to a cage reared, experimentally naive animal the situation is unfamiliar, its novelty may be presumed to provoke a fear or anxiety type of reaction (Doyle and Yule, 1959). Ivinskis (1970) has noted that it is usually difficult to estimate the validity of open field measures, mainly because there are no external validation criteria.

The two most commonly used measures of emotionality are ambulation and defecation. Hall (1934, 1936) first established the validity of defecation scores as a measure of open field emotionality. Subsequent researchers have replicated his findings (eg. Ivinskis, 1970; Whimbey and Denenberg, 1967). High defecation rates correlate positively with emotionality. As well as having proven validity, the reliability of this measure has been shown to be acceptable in several species.

Like defecation, ambulation shows acceptable reliability across species. A negative relationship between the two measures has consistently been shown within the literature (Walsh and Cummins, 1976). This suggests that ambulation would increase with decreased emotionality. However, both increases and decreases have been observed over time. It has been suggested that ambulation could provide a factorially complex score, indicative of an emotionality factor and an exploration factor. Thus, rather than reflecting a decrease in emotionality, ambulation could reflect an increase in exploratory drive. The relationship between exploration itself and emotionality is unclear. High levels of emotionality may inhibit exploration as is seen in its extreme in the freezing response. Here the animal "adopts a compact crouching posture and remains completely motionless, even nose twitching being absent" (Doyle and Yule, 1959, p.18). Alternatively, emotionally arousing stimuli may induce exploration if, as suggested by Glanzer (1953), the biological function of exploration is to reduce one's fear of the environment. The interpretation of an ambulation measure is difficult, especially where only one trial is given and defecation score information is not considered (Walsh and Cummins, 1976). Although providing an apparently reliable measure of emotionality, ambulation's validity has been questioned. Ivinskis (1970), on the basis of three criteria; 1) inter-trial decrease of scores across four days; 2) effects of prior open field testing to decrease scores, and 3) effects of alterations in ambient auditory and visual stimulation, concluded that ambulation was an invalid measure of emotionality. However, the adequacy of these criteria would seem to be debatable. Ivinskis concluded that only the third was satisfactory.

Like ambulation, rearing has at times been taken as a negative indicator of emotionality (Ader, 1965; Gray, Levine and Broadhurst, 1965). If this is so, then a negative relationship between defecation

and rearing should occur over retests. Ivinskis (1970) failed to find such a relationship. If rearing was considered to measure exploratory behaviour (Pare, 1964) Ivinskis suggested that the decrease in rearing scores over retests could be explained more satisfactorily. Results of this experiment agreed with those of Montgomery (1953) who found that exploration decreased over time with repeated exposure to the same environment.

Ambulation and emergence latency are negatively correlated in the rat. Ivinskis (1970) found latency to be a valid measure of emotionality. According to Beveridge, Meatchem and Kirk (1981), although a number of tests exist (eg. the open field), the emergence test is generally thought to best represent an adequate measure of the varying degrees of emotionality in laboratory rodents. Emergence latency increases with increased emotionality and decreases with decreased emotionality. Time taken to emerge also shows some loading on the exploratory behaviour factor.

Grooming displayed in an unfamiliar environment is generally regarded as a sign of fear (Tinbergen, 1952). Some authors disagree. For example Bolles (1960) suggested that, rather than being a reaction to novelty or an anxiety/fear provoking situation, much of the time grooming occurs because a sequence of eating, drinking or exploratory behaviour has just been concluded. This grooming is highly stereotyped - licking the fur, scratching with a hind leg, and washing the face. It may be regarded as displacement activity differing from other types of grooming such as those occurring in association with sexual and maternal behaviour. Doyle and Yule (1959) found no support for grooming as a displacement reaction in the open field. Also, the grooming behaviour of the male albino rats used as subjects was not correlated with emotionality as measured by, for example, defecation. Walsh and Cummins (1976) noted that grooming has been found to be of relatively low reliability. It may be this which accounts for the inconsistent findings in the literature.

In the open field rats are reluctant to leave the wall or corners and approach the centre. Occupancy of the periphery, either near walls (Valle, 1970) or in corners (Morrison and Thatcher,1969) has therefore been used as a measure of timidity.

Aims of the present study

The main aim of this study was to determine whether three of the beta-adrenoceptor blocking drugs exert any behavioural effects in a free responding situation and whether such effects could be the result of an anxiolytic action. It has been suggested that observation of unconditioned behaviour, in the emergence test and open field for example, could provide a useful way of highlighting subtle drug effects which might be obscured by more rigorous experimental procedures (Maxwell, 1968).

A further aim was to determine whether any sex differences in responsiveness to the drugs exist. Many past investigations used only male rats or mice as subjects.

CHAPTER TWO

EXPERIMENT ONE

METHOD

Subjects

Forty-two randomly derived Wistar strain albino rats, 13 male (mean age 114 days; range 111-117 days) and 29 female (mean age 112 days; range 109-115 days) from the Otago Medical School were used. Five males (age 94 days) and one female (age 130 days) from the University of Canterbury colony were added to make the total number of test animals 48. Housing was in group cages of three or four same-sex animals. The rats had been handled regularly from birth. An optimal temperature of 24±1°C was aimed for in the colony room. Due to inconsistencies within the heating system variation of ±5°C occurred. A reversed 12 hour light-dark cycle was in operation, the dark phase lasting from 6 am to 6 pm. A noise level of 52-70dB was characteristic of the colony room. All animals received ad lib food and water up until the time of individual testing. Testing was conducted between 1-4.30 pm.

Apparatus

Subjects were tested for emergence latency and behaviour in the open field. The emergence test apparatus consisted of a darkened wooden box separated by a sliding partition from a larger, illuminated box. The

darkened box, lxwxh = 210x175x310mm, had a hinged, semi-transparent lid through which each experimental subject was introduced to the test apparatus. Removal of the partition revealed a 100x100mm opening into the illuminated area. Walls of the illuminated area were wooden, painted white. The floor was made of translucent white perspex. Lighting of 375 lux was provided by a source situated beneath the perspex. A wire mesh lid covered the area. This was painted white in order to reflect light back into the enclosed space. This space had dimensions lxwxh = 525x525x335mm.

The open field apparatus had clear perspex walls 300mm high. The base, 600x600mm, was black perspex, being divided by white lines into 16 150x150mm numbered squares.

Within the experimental room white noise was maintained at a level of 60dB. Illumination was 320 lux. A beeper, set to sound at five second intervals, was on continuously.

Procedure

Animals were randomly assigned to one saline and three drug groups in the ratios

	saline	5mg/kg	10mg/kg	20mg/kg
males	5	4	5	4
females	7	8	7	8

Subjects were tested in series of three, six animals being tested per day. In the colony room rats were transferred by the experimenter from their home cages to holding cages, either alone or with age mates from the same home cage if these were also being tested. The holding cages were then carried to the experimental room approximately 5m away. At 15 minute intervals a rat was removed from its holding cage, injected (i.p.)

with either isotonic saline (0.1cc/kg) or propranolol, 5, 10 or 20mg/kg. Because only the 5mg/kg concentration was available volume changes were required for different doses.

5mg/kg - 1cc/kg

10mg/kg - 2cc/kg

20mg/kg - 4cc/kg

The rat was then returned to its holding cage. Approximately 30 minutes after injection (range 30-36 minutes) the subject was placed by the experimenter in the darkened box of the emergence test apparatus 2m away. Beveridge et al. (1981), using confinement times of 15, 30 and 60 sec, found a significant linear relationship between emergence latency and confinement time. Increased length of confinement in the darkened box was associated with longer latencies. For this reason a confinement time of 15 sec was chosen for this experiment. After 15 seconds the wooden partition was removed and time taken for the rat to move into the illuminated area was recorded. The experimenter observed emergence through the wire mesh. Two emergence measures were recorded for each animal, ie.,

(a) Time taken for all parts of a forward facing rat, up to and including the point halfway between the front and rear legs (as judged by the experimenter) to emerge.

(b) Time taken for all parts of a forward facing rat, up to and including the rear legs, to emerge.

If the subject had not emerged after 5 minutes timing ceased and latency was recorded as 300 seconds.

Upon completion of the emergence test the rat was picked up by the experimenter and placed in the centre of the open field apparatus. The centre was chosen because of the tendency noted by Satinder (1969) of rats to remain on the side of the field where they were originally placed. Each subject was observed for 10 minutes. Every five seconds,

as indicated by the automatic beeper, it was noted which square the animal was predominantly in. Also noted were

(a) rearing behaviour, defined as the rat standing, either supported or unsupported, on its hind legs

(b) walking, either within or between squares

(c) grooming, defined as preening the body with either the mouth or leg(s).

At the end of each trial faecal boli were removed and counted. Both the inside of the open field and the darkened box of the emergence test apparatus were thoroughly washed with a solution of "Hibitane" disinfectant/masker. It may have been unnecessary to wash the darkened box since Beveridge et al. found no specific effect of conspecific odour on emergence latency when confinement time was 15 seconds. However, a significant effect was found with 60 second latency.

Total occurrences of each behaviour (walking, rearing, grooming) recorded with successive 200 sec intervals of the 10 minute open field test were determined for each rat. From records of squares occupied movement from one square to another in adjacent 5 sec intervals was recorded as a 'transition'. Total numbers of transitions in successive 200 sec intervals were calculated from these. The number of recordings during which the subject occupied corner squares (1, 4, 13 or 16) or centre squares (6, 7, 10 or 11) were also calculated for each 200 sec interval.

RESULTS AND DISCUSSION

For each of the behavioural measures (walking, rearing, grooming, centre squares, corner squares, transitions, half body emergence, total body emergence) separate 2x4 Analyses of Variance were used to evaluate the effects of the 4 doses (saline, 5, 10 and 20mg/kg drug) for the 2

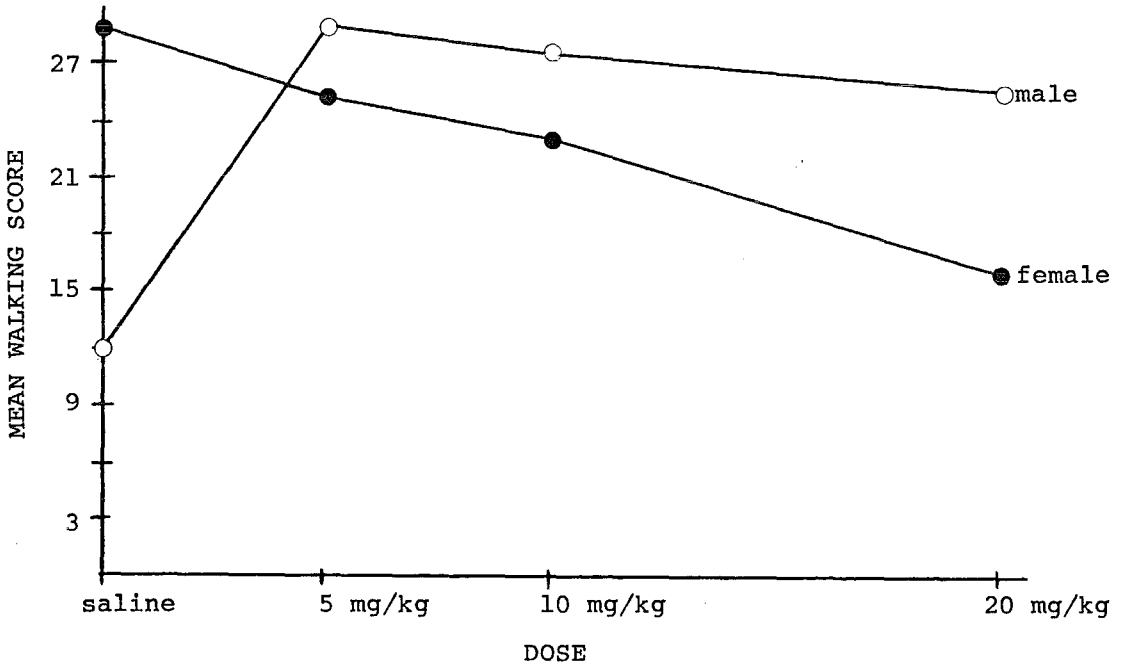
sexes. Where significant dose or interaction effects occurred individual group means were compared by post-hoc t-tests. For each t-test MS errors comprised the estimate of variance (df=40). Eyeballing the raw data suggested that analysis of the faecal boli measure would provide little useful information. This was because the data showed high variability between and within treatment groups. Results of the statistical analyses are summarised in Appendix A.

Walking. Neither main effect was significant at the .05 level but a significant sex-dose interaction occurred (illustrated in Figure 1; $F_{AB}(3,40) = 5.56$, $p<.01$). For males there were no significant differences in mean walking scores between the different drug doses. Drugged animals showed significantly higher mean walking scores than saline controls ($p<.01$ (5mg/kg), $p<.01$ (10mg/kg), $p<.05$ (20mg/kg)). Propranolol had the opposite effect on females. The mean walking scores of drugged animals were less than that of the saline group although only the 20mg/kg rats showed a decrease which was significant ($p<.01$).

The current results disagree with the U-shaped dose response curve obtained by Engel and Liljequist (1976) who investigated the effect of dl-propranolol on ethanol induced locomotor stimulation in female mice. Maximum reduction of induced locomotor stimulation occurred at doses of 0.5-2mg/kg. The reduction disappeared with doses greater than 10mg/kg.

Figure 1

Propranolol dose-sex interaction effect on walking scores



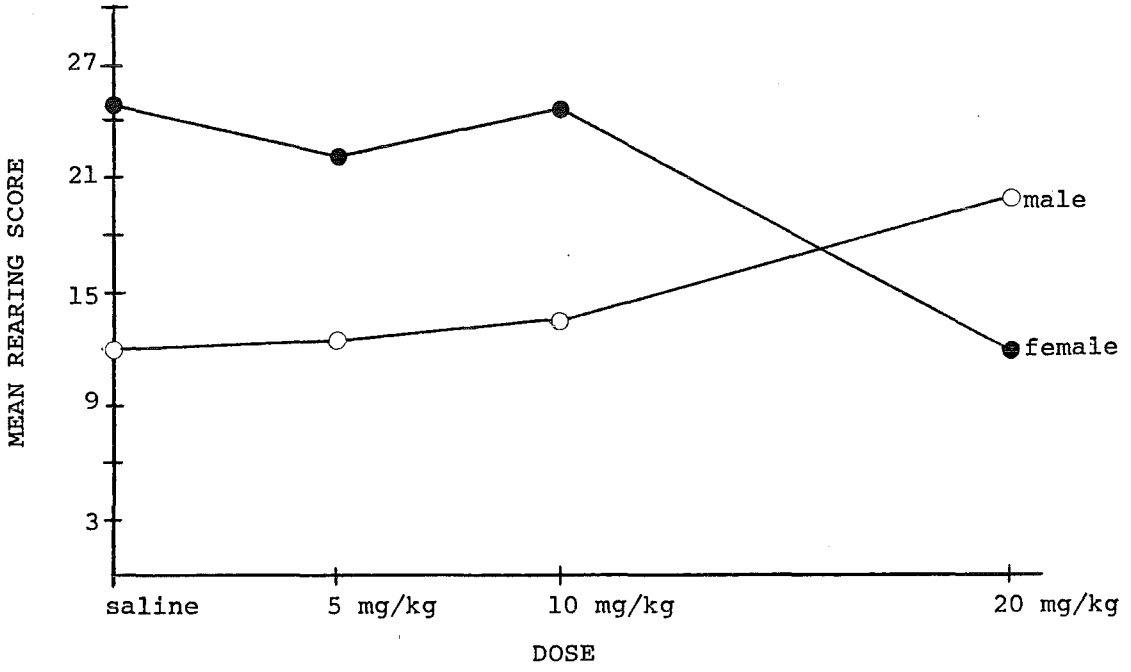
Rearing. Figure 2 illustrates both the significant sex main effect ($F_A(1,40)=5.67$, $p<.05$) and the significant sex-dose interaction ($F_{AB}(3,40)=3.14$, $p<.05$). Propranolol did not affect the rearing scores of the males, all comparisons were non significant. Female rats injected with 20mg/kg propranolol showed mean rearing scores which were significantly less than those of females in the saline, 5mg/kg and 10mg/kg groups. The otherwise nonsignificant changes in rearing rates between different groups were similar to results reported by Weinstock and Speiser (1973). Doses less than 20mg/kg were ineffective in decreasing the rearing of group housed control male rats. The aim of the experiment was to investigate the effect of propranolol on isolation induced hyperactivity.

The significant sex difference in rearing in the present experiment saw females with a greater average rearing score. The mean rearing score for females was 21.09 while for males it was 14.51. Hitchcock (1925) found that female albino rats are usually more active

than males. The significant sex difference in the present experiment is in agreement with those findings. It is also in agreement with Hughes (1972) who found, more specifically, that rearing scores for female albino rats are greater than those of males.

Figure 2

Propranolol dose-sex interaction effect on rearing scores



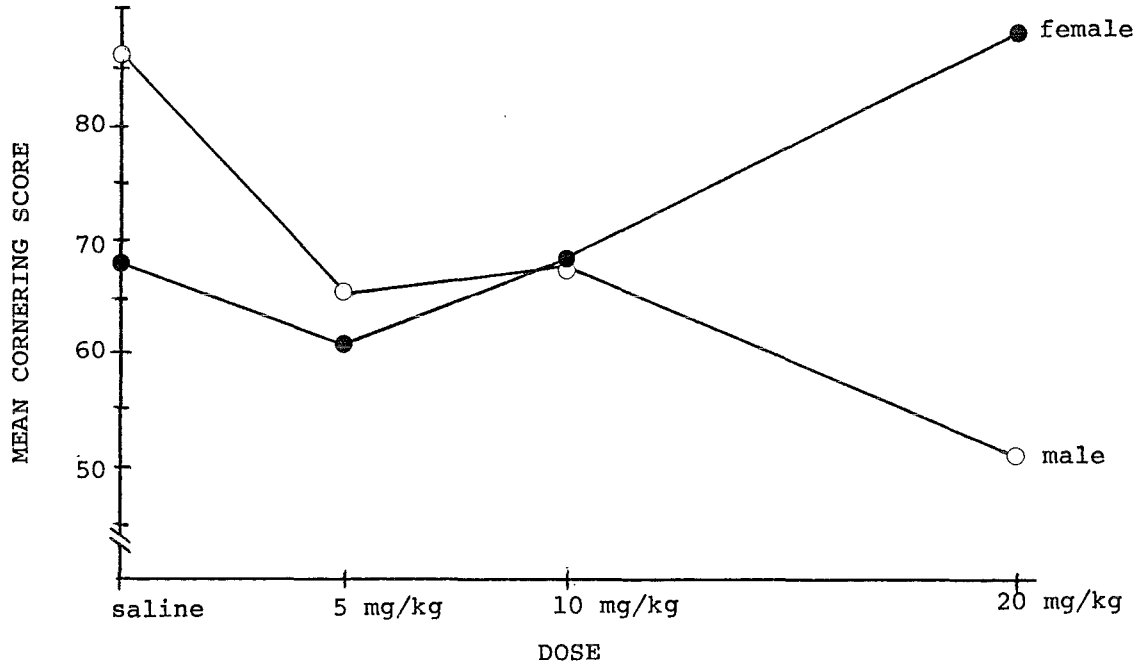
Grooming. Both main effects and the interaction effect were non significant. Searle (1983) also found no effect of propranolol on the grooming behaviour of rats in the open field (in the absence of a noxious stimulus). The tendency in the current results was towards a decrease in grooming behaviour with increased dose for both males and females.

Transitions. All effects were non significant. Results were similar to those of Ksiazek and Kleinrok (1974) who reported no significant dose effect on transitions using 0.4, 1.0 and 2.0 µmole propranolol. At these dose levels any antianxiety effect is being confounded by a toxicity effect. The authors reported that at a dose of 2.0 µmole 4 out of ten subjects died.

Corners. The sex-dose interaction effect was significant for this variable ($F_{AB}(3,40)=3.24$, $p<.05$). The relationship is represented by Figure 3. Both main effects were non significant. For females the 20mg/kg group showed a significant increase in cornering behaviour in comparison to the 5mg/kg group. All other comprisons were non significant. Drugged males showed a decrease in cornering behaviour in comparison to the male saline group. Only the 20mg/kg group showed a decrease which was significant.

Figure 3

Propranolol dose-sex interaction effect on corner scores



Centres. Neither main effect, nor the interaction effect were significant. Centre and cornering behaviours were mutually exclusive in this experiment. Reflecting this, males tended to be found more in the centre of the open field with increasing drug dose while females tended to be there less.

Half body emergence. Although neither the main effects nor the interaction effect were significant a U shaped dose response relationship was observed for the half body emergency measure. Emergence latency was lowest at a dose of 5mg/kg propranolol.

Total body emergence. All effects were non significant. For males the U shaped dose response curve observed in the half body emergence measure remained. Non significance of the gender main effect in the emergence test was also found by Beveridge et al. (1981).

To summarise the results of Experiment One, 20mg/kg propranolol significantly decreased the rearing and walking of female rats in comparison to control subjects. This group also showed significantly increased cornering behaviour in comparison to the 5mg/kg female group. Unless some property of propranolol itself is increasing emotionality this suggests a sedative effect. The transitions measure provides a useful indicator of activity since increase emotionality has been related to decreased activity (Ader, 1965; Walsh and Cummins, 1976). Although insignificant the trend was towards decreased transition scores with increasing drug doses for females, again suggesting sedation ($\underline{M}$ = 65 (saline), 62.75 (5mg/kg), 63.86 (10mg/kg) and 50.75 (20mg/kg)). Bainbridge and Greenwood (1971), for example, noted such a sedating or tranquillizing effect of 10 and 20mg/kg propranolol on male albino rats conditioned to expect electric shock. This sedation was interpreted as an antianxiety effect because an increase in grooming behaviour followed conditioned stimulus presentation. The results for the grooming measure in the present experiment were insignificant so such a conclusion cannot be drawn. Alternatively the significant results for 20mg/kg females could indicate a reduction in exploratory drive by propranolol.

Significant results for males occurred with the walking measure. Drugged males had mean walking scores significantly greater than controls. If ambulation is interpreted as increasing with decreasing emotionality it would be possible to say that propranolol reduces emotionality in male rats. Some support for this interpretation comes from the significant decrease in cornering behaviour, an indicator of timidity, of the 20mg/kg male group in comparison to the saline group. However, there are alternative interpretations of the walking measure (increased exploratory drive or general activity) and, as suggested by Walsh and Cummins (1976), in the absence of validation from other measures such as defecation, firm conclusions cannot be drawn.

For grooming, transitions, centres, half body emergence and total body emergence the interaction effect and main effects were insignificant.

CHAPTER THREE

EXPERIMENT TWO

METHOD

Subjects

Forty-eight albino rats, randomly derived Wistar strain, 18 males (mean age 120 days; range 117-124) and 30 females (mean age 119 days; range 115-123) from the Otago Medical School colony were used. These animals were reared and maintained under identical conditions to those of Experiment One.

Apparatus

The same emergence test and open field apparatus described in Experiment One were used.

Procedure

Both sexes were randomly assigned to one saline and three drug conditions in the same ratios as Experiment One. The experimental procedure followed exactly that of Experiment One with the exception of drug administration. Animals were injected (1cc/kg i.p.) with isotonic saline or oxprenolol (5, 10 or 20 mg/kg).

RESULTS AND DISCUSSION

As in Experiment One, 2x4 Analyses of Variance followed by t-tests were used to evaluate the data. Results of the statistical analyses are summarised in Appendix B. Boli measures were again not analysed due to high variability between and within treatment groups.

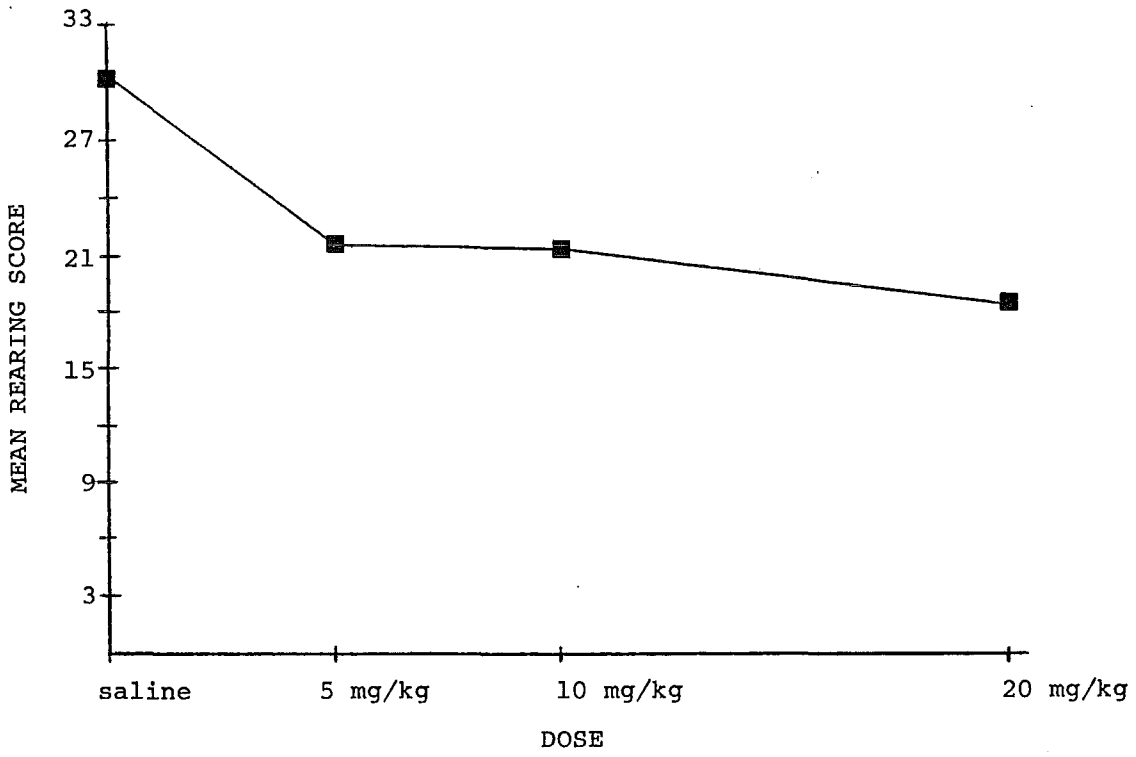
Walking. Neither main effect was significant. The sex-dose interaction was also insignificant. The results are similar to those of Hughes (1981) and Noble and Delini-Stula (1976). Hughes (1981) found that oxprenolol (10 and 20mg/kg i.p.) had no effect on the ambulation of male and female hooded rats in an exploration box. Noble and Delini-Stula (1976) found that oxprenolol (100mg/kg p.o.) had no effect on exploratory behaviour which had been suppressed by previous exposure to inescapable electric shock. This experiment involved male Wistar rats subjected to repeated measures over 5 days.

Rearing. There was a significant sex main effect ($F_A(1,40)= 5.99$, $p<.05$) as well as a significant dose main effect ($F_B(3,40)=4.36$, $p<.01$) on rearing behaviour. Figure 4 shows the relationship between dose of oxprenolol and rearing. Saline treated rats showed significantly greater mean rearing scores than any of the oxprenolol treated groups ($p<.05$ (5mg/kg), $p<.05$ (10mg/kg), $p<.01$ (20mg/kg)). All other comparisons were non significant. The significant dose main effect contrasts with the findings of Hughes (1981). In that investigation oxprenolol (10 and 20 mg/kg i.p.) had no effect on the rearing behaviour of male and female hooded rats in comparison to saline controls.

In the present experiment the significant sex difference favoured females with the greater mean rearing score of 26.46 while males scored 19.89.

Figure 4

Oxprenolol dose main effect upon rearing scores

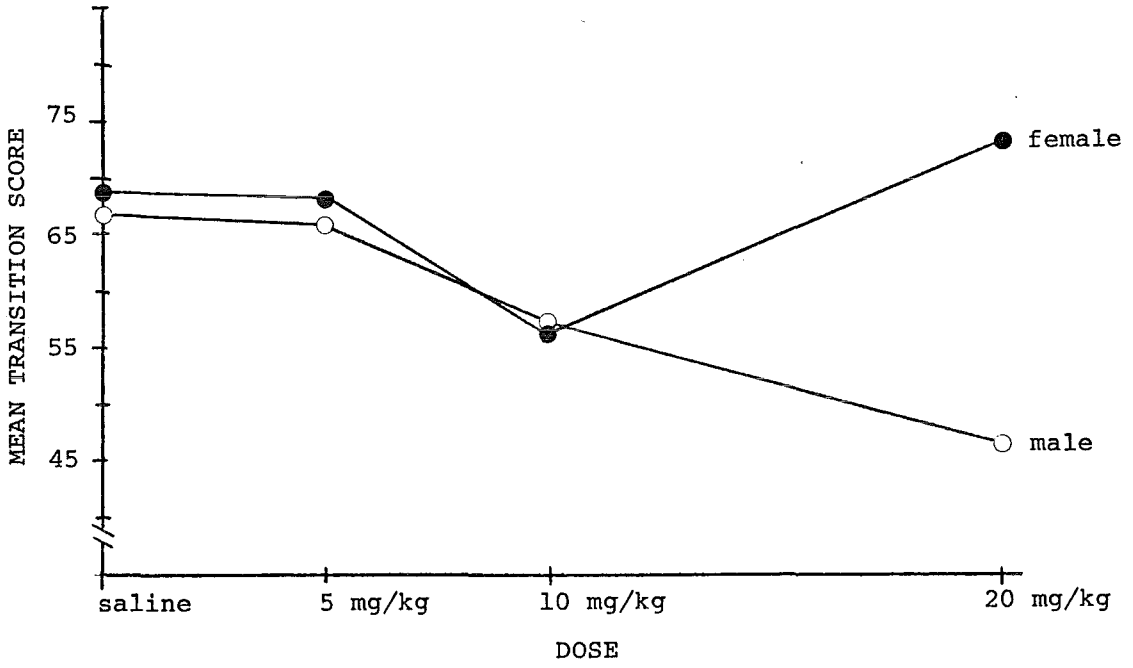


Grooming. There were no significant effects on this variable.

Transitions. For this variable neither the dose nor the sex main effect was significant. The interaction effect (outlined in Figure 5) was significant ($F_{AB}(3,40)=2.88$, $p<.05$). The male subjects in the 20mg/kg group showed a decrease in mean transition scores which was significantly less than that of the saline and 5mg/kg groups. Females in the 20mg/kg group showed an increase which was significantly greater than that of the 10mg/kg group. For both sexes all other comparisons were non significant.

Figure 5

Oxprenolol dose-sex interaction effect on transition scores



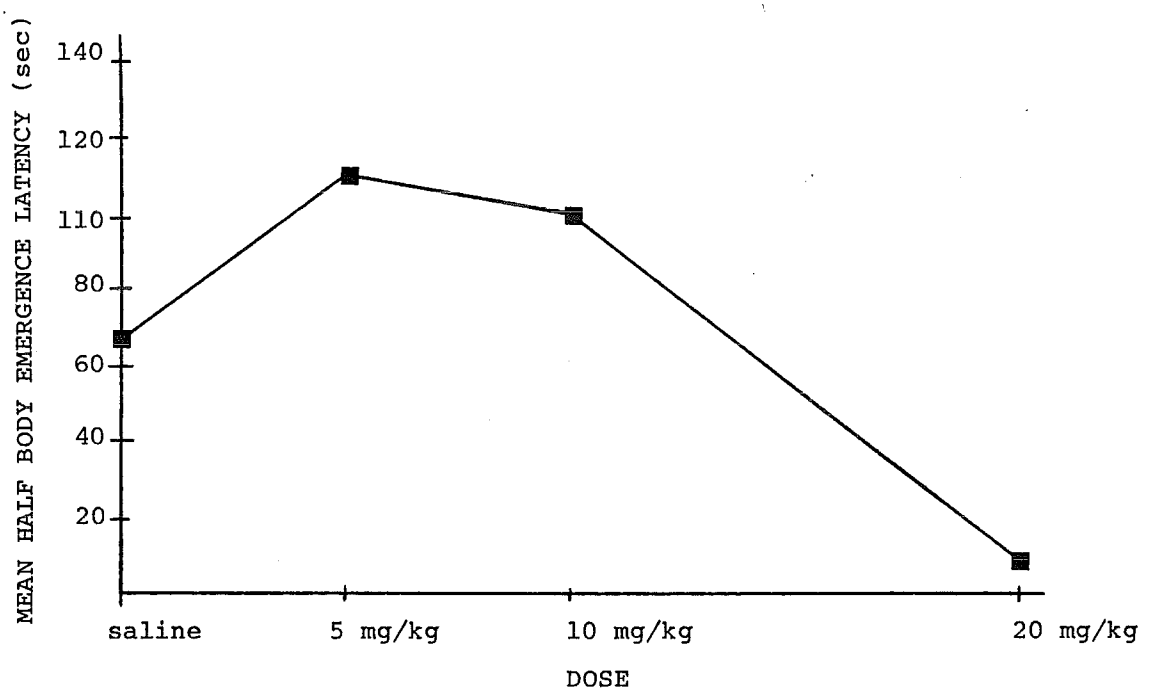
Corners. All effects were non significant. Although reaching a significance level of 0.10, the dose main effect just failed to reach an α level of .05. Directional similarities between oxprenolol and propranolol were apparent in the dose response curve for females.

Centres. Neither the main effects nor the interaction effect were significant. Once again there were directional similarities in the dose response curves of oxprenolol and propranolol. The similarities extended to males as well as females.

Half body emergence. Figure 6 illustrates the significant dose main effect ($F_B(3,40)=3.04$, $p<.05$). The sex main effect was also significant for this variable ($F_A(1,40)=4.75$, $p<.05$). Females showed an average emergence latency of 44.44 sec while for males it was 103.10 sec. Post hoc t-tests showed that rats in the 20mg/kg groups emerged significantly faster that those in the 5 and 10mg/kg groups.

Figure 6

Oxprenolol dose main effect on half body emergence latency

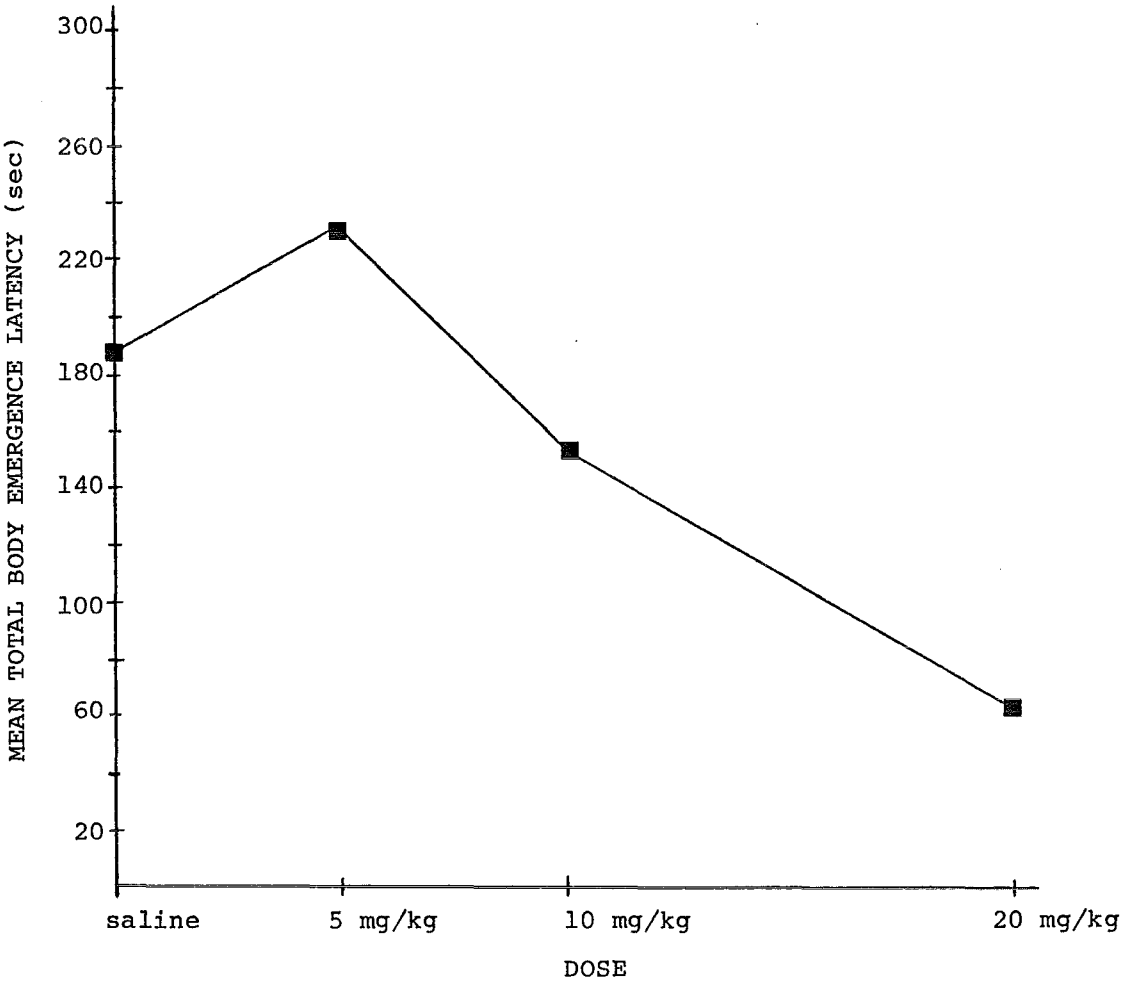


Total body emergence. As with the half body emergence measure both the sex main effect ($F_A(1,40)=4.7$, $p<.05$) and the dose main effect were significant ($F_B(3,40)=4.78$, $p<.01$). A similar dose response relationship was obtained to the half body emergence measure. This might be expected since the measures are not independent. Figure 7 shows the relationship between oxprenolol and total body emergence. The 20mg/kg dose was associated with a reduction in total body emergence latency significantly below that of the saline, 5mg/kg and 10mg/kg groups. All other comparisons were non significant. The significant difference between the saline and 20mg/kg group agrees with that found by Hughes (1981). Hughes noted that both 10 and 20mg/kg oxprenolol significantly shortened the emergence latencies of hooded rats in comparison to controls. Methodological differences could account for the differing results in the 10mg/kg groups. Hughes' subjects were experiencing the effects of oxprenolol for the second time, having been injected one week previously with the same dose.

For the total body emergence measure females showed a mean emergence latency of 119.24 sec in comparison to 190.2 sec for males.

Figure 7

Oxprenolol dose main effect on total body emergence latency



To summarise the results of Experiment Two, significant dose main effects occurred for rearing, half body emergence and total body emergence measures. Rats treated with 20mg/kg oxprenolol showed significantly shorter mean total body emergence times than animals in other groups. This could indicate decreased emotionality in the 20mg/kg subjects. However, there was no significant support for this interpretation from walking, grooming, corners or centre measures. 20mg/kg rats had

significantly shorter mean half body emergence latencies than the 5 and 10mg/kg groups but not the saline group. How this result should be interpreted is unclear.

Drugged animals reared significantly less than saline controls. If rearing is inversely related to emotionality (Ader, 1965; Gray, Levine and Broadhurst, 1965) this suggests, in contrast to results from the total body emergence measure, that oxprenolol increased emotionality. Alternatively the rearing results could be interpreted in terms of decreased exploratory behaviour (Pare, 1964) or sedation due to oxprenolol administration. Once again there was no significant support for these interpretations from other measures such as walking and emergence latencies. Some validation was obtained from within the significant sex-dose interaction effect on transition behaviour. 20mg/kg males recorded significantly fewer transitions than saline and 5mg/kg males (20mg/kg females showed the opposite tendency, recording significantly more transitions than the 10mg/kg group).

Significant sex main effects were obtained for rearing, half body emergence and total body emergence measures. For all these variables females showed the higher level of activity. With respect to walking, grooming, corners and centres measures, all effects were insignificant.

CHAPTER FOUR

EXPERIMENT THREE

METHOD

Subjects

Forty-eight albino rats, randomly derived Wistar strain, 18 males (mean age 130 days; range 125-136) and 30 females (mean age 124 days; range 117-129) from the Otago Medical School colony were used. These animals were reared and maintained under identical conditions to those of Experiments One and Two.

Apparatus

The same emergence test and open field apparatus described in Experiments One and Two were used.

Procedure

Procedural details followed those of Experiments One and Two with the exception of drug administration. Animals were injected (2cc/kg i.p.) with isotonic saline or atenolol (5, 10 or 20mg/kg).

RESULTS AND DISCUSSION

As in Experiments One and Two, 2x4 Analyses of Variance followed by t-tests were used to evaluate the data. Results of the statistical analyses are summarised in Appendix C. Boli measures were not included in the analysis because of high between- and within-group variability.

Walking. Only the sex main effect was significant ($F(1,40)=8.75$, $p<.01$). The sex difference was in the expected direction, with females exhibiting greater activity than males. The mean walking score for females was 20.95 and for males was 15.34.

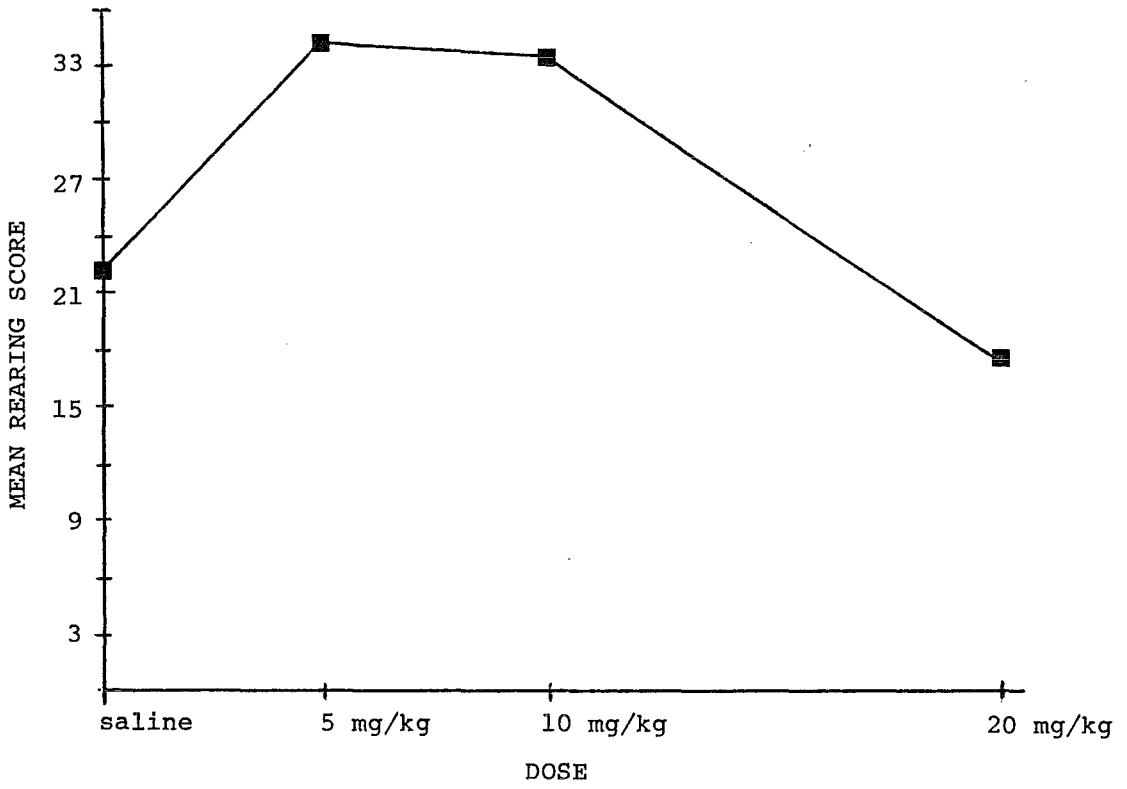
Rearing. For this variable both the sex main effect ($F_A(1,40)=5.01$, $p<.05$) and the dose main effect were significant ($F_B(3,40)=5.17$, $p<.01$). The dose main effect is shown in Figure 8. Both the 5 and 10mg/kg groups showed mean rearing scores significantly greater than the saline and 20mg/kg groups. The significant sex difference once again showed females exhibiting greater rearing activity than males. The average rearing score for females was 31.07, that for males was 22.86.

Grooming. Neither main effect nor the interaction effect was significant.

Transitions. Only the sex main effect was significant ($F_A(1,40)=22.5$, $p<.01$). The sex differrence was once again in the expected direction, with females showing greater activity. The average transition score for females was 75.86 compared to 49.73 for males.

Figure 8

Atenolol dose main effect on rearing scores



Corners. There were no significant effects for this variable. Although non significant, there was a trend towards increasing cornering behaviour with increasing drug dose.

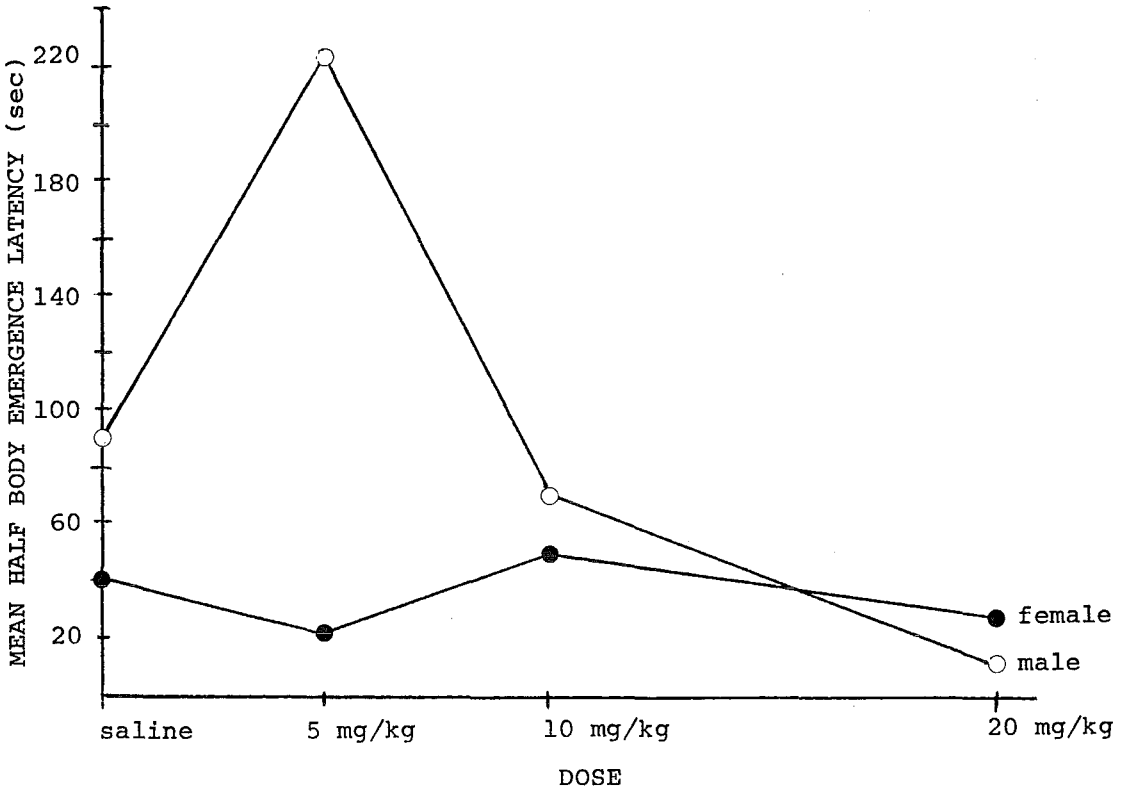
Centres. Once again there were no significant effects. Reflecting the association of this variable with the previous one (ie. the behaviours are mutually exclusive) a trend towards decreasing centering behaviour with increasing drug dose was observed ($\underline{M}$=19.97 (saline), 22.13 (5mg/kg), 15.37 (10mg/kg), and 7.62 (20mg/kg).

Half body emergence. Figure 9 illustrates the significant interaction effect for atenolol ($F_{AB}(3,40)=3.47$, $p<.05$). The sex main effect was also significant ($F_{A}(1,40)=6.49$, $p<.05$). Males showed a mean emergence time of 102.3 sec, for females it was 36.64 sec.

Differing doses of atenolol significantly affected the half body emergence times of males only. The 5mg/kg group showed a mean emergence time significantly greater than all other groups ($p<.05$ (saline), $p<.05$ (10mg/kg), $p<.01$ (20mg/kg)). This increase at the 5mg/kg dose is inexplicable and suggests that some extraneous variable is affecting the group. Since all the animals in this group (N=4) were tested on the same day it is possible that some feature of the environment affected behaviour, for example a temperature fluctuation. However, a similar increase in emergence time is not obvious in the 5mg/kg female group, two of whom were tested on the same day as the males.

Figure 9

Atenolol dose-sex interaction effect on
half body emergence latency



Total body emergence. Although none of the effects were significant trends were similar to those found in the half body emergence measure.

In summary, results of Experiment Three indicated that females showed significantly greater activity levels than males for the walking, rearing, transitions and half body emergence measures. For rearing the dose main effect was also significant. Rats injected with 5 and 10mg/kg atenolol gained significantly greater mean rearing scores that those in the saline and 20mg/kg groups. Such an inverted U shaped dose response curve has no obvious interpretation. One could speculate that at doses of 5 and 10mg/kg atenolol either potentiates exploration or general activity or decreases emotionality. In contrast, at 20mg/kg the dose takes on noxious properties, inhibiting activity or increasing emotionality to levels experienced by the saline group.

The interaction effect was significant for the half body emergence measure. 5mg/kg males took significantly longer to emerge than males in other groups. As previously mentioned, such a result was probably due to some unexplained external influence on the subjects in this group.

Main effects and interaction effects were insignificant for the grooming, corners, centres and total body emergence measures.

CHAPTER FIVE

GENERAL CONCLUSIONS

Results for the three drugs propranolol, oxprenolol and atenolol showed more differences than similarities. It would seem unlikely, therefore, that the blockers used in these experiments exerted their effects via the same mechanism of action. Had Experiments One, Two and Three produced equivalent results it might have been appropriate to conclude that any anxiolytic effects exerted by propranolol (and oxprenolol) were peripherally mediated since, although all three drugs show peripheral activity, atenolol shows very little activity within the centrel nervous system. Some evidence for a centrally mediated drug effect came from the results obtained for female subjects treated with propranolol (decreased rearing and walking, increased cornering). These results could be interpreted in terms of a drug related decrease in exploratory behaviour or a sedation effect, both of which are under central nervous control. Alternatively, as previously mentioned, some property of the drug itself may have been aversive to the subjects, resulting in increased emotionality. If the significant effects on male subjects (increased walking, decreased cornering) given propranolol were interpreted with an exploratory drive framework it would be possible to conclude that, here also, the drug was having its effect on central processes. The alternative interpretation of these results is in terms of an antianxiety effect, which may or may not include an influence on exploratory behaviour. Since the results of this group differed from those obtained for subjects treated with atenolol it may be valid to

suggest that propranolol's effect was centrally mediated. Such a result would be in agreement with the majority of animal research.

The significant drug related decreases in rearing obtained with oxprenolol show similarities to those found in female subjects treated with propranolol. As such, there is the possibility that oxprenolol's effect was central. Decreased drug-related rearing does not suggest that the effect was an anxiolytic one. Such a result is more in keeping with a decrease in exploratory drive or sedation. Support for this interpretation comes from the transition scores of males treated with oxprenolol. Transition scores decreased with increasing drug dose. Alternative conclusions could be drawn from the emergence measures. Here, decreased emergence latencies associated with 20mg/kg oxprenolol could indicate an antianxiety effect of the drug.

Results for atenolol were for the most part insignificant, although 20mg/kg subjects showed significantly less rearing behaviour than either 5 or 10mg/kg rats, but not saline controls. Such a result differs from both those obtained from propranolol and oxprenolol and may be attributable to an alternative mechanism of action of the drug.

Sex differences in behaviour were apparent in all three experiments. The direction of these differences with respect to activity levels was in agreement with past research (Hitchcock, 1925). Females exhibited higher activity levels than males. Sex differences in drug effects showed less consistency. While female rats treated with 20mg/kg propranolol walked significantly less than controls, 20mg/kg males walked significantly more. 20mg/kg treated females engaged in significantly more cornering behaviour than 5mg/kg females. 20mg/kg males cornered significantly less than controls. Similarly, females injected with 20mg/kg oxprenolol recorded significantly more transitions than the 10mg/kg groups, while 20mg/kg males had transition scores significantly less than 5mg/kg and control males. Such differential effects, although

not open to obvious interpretation, were not apparent in past investigations, the majority of which used male subjects alone. Sex differences in reactivity to the β blockers have not been a feature of the literature on human subjects either. However, a comparison of results may be inappropriate since, as previously noted, variations in blocker effects occur across species. The differences could provide a focus of future research in order to determine whether they were an artefact of this investigation or a true result of drug administration.

The present investigation used three drug doses and a control saline group in each experiment. A greater range of doses may have provided more information about dose-response curves. Doses of 100mg/kg and more have been used in the past so conclusions from the current research are based on drug concentrations at the lower end of the scale. Upon equilibration across the blood-brain barrier more actual drug is found within the central nervous system when higher doses are administered peripherally. With β blockers such as oxprenolol and atenolol greater concentrations could result in a significant effect on central β receptors. For example, Noble and Delini-Stula (1976) found decreased grooming behaviour over trials with 100mg/kg oxprenolol administered orally to male Wistar rats. In Experiments One, Two and Three no drug had a significant effect on grooming. Possibly this was because no retesting occurred. Alternatively this may have been because doses were not high enough for a centrally-mediated effect on grooming to be observed. Future research should therefore look at a wider range of doses in order to obtain a clearer picture of dose-response relationships.

Conclusions from this study are limited by the relationship of the variables measured to the underlying concepts of emotionality, exploratory behaviour and general activity. It is unlikely, however, that more discriminative variables will be developed since the concepts

they are measuring can only be inferred, not quantified directly. For this reason also it is difficult to compare the emotional effects of the β blockers on rats (and mice) to those on humans. It may be unrealistic to expect, therefore, that animal research will clarify the clinical picture of β blocker action.

ACKNOWLEDGEMENTS

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APPENDICES

APPENDIX A

Summaries of Statistical Analyses for Experiment One

Note: * $p<.05$; ** $p<.01$

t-test comparisons were carried out in order to distinguish significant differences in group means. Comparisons were based on graphical differences between group mean points along the Y axis (ie. those points which were the greatest distance apart were compared first).

Once distances between means yielded insignificant differences no further comparisons were carried out.

Table 1

ANOVA Summary Table: Walking Measure

Source of Variation	SS	df	MS	F
A (sex)	0	1	0	0
B (dose)	366.17	3	122.06	1.74
AB	1171.20	3	390.40	5.56**
error	2808.19	40	70.20	

Table 2

ANOVA Summary Table: Rearing Measure

Source of Variation	SS	df	MS	F
A (sex)	481.92	1	481.92	5.67*
B (dose)	69.63	3	23.21	0.27
AB	800.24	3	266.75	3.14*
error	3402.41	40	85.06	

Table 3

ANOVA Summary Table: Grooming Measure

Source of Variation	SS	df	MS	F
A (sex)	470.28	1	470.28	3.30
B (dose)	793.95	3	264.65	1.86
AB	112.85	3	37.62	0.26
error	5704.87	40	142.62	

Table 4

ANOVA Summary Table: Transitions Measure

Source of Variation	SS	df	MS	F
A (sex)	426.83	1	426.83	1.01
B (dose)	182.92	3	60.97	0.14
AB	2162.89	3	720.96	1.71
error	16893.06	40	422.33	

Table 5

ANOVA Summary Table: Corners Measure

Source of Variation	SS	df	MS	F
A (sex)	112.51	1	112.51	0.24
B (dose)	1137.95	3	379.32	0.81
AB	4524.62	3	1508.21	3.24*
error	18636.02	40	465.90	

Table 6

ANOVA Summary Table: Centres Measure

Source of Variation	SS	df	MS	F
A (sex)	4.01	1	4.01	0.10
B (dose)	69.24	3	23.08	0.59
AB	284.57	3	94.86	2.44
error	1556.48	40	38.91	

Table 7

ANOVA Summary Table: Half Body Emergence Measure

Source of Variation	SS	df	MS	F
A (sex)	4859.05	1	4859.05	0.50
B (dose)	71737.42	3	23912.47	2.47
AB	13592.03	3	4530.68	0.47
error	387307.14	40	9682.68	

Table 8

ANOVA Summary Table: Total Body Emergence Measure

Source of Variation	SS	df	MS	F
A (sex)	24248.33	1	24248.33	2.3
B (dose)	21606.75	3	7202.25	0.68
AB	43073.65	3	14357.88	1.36
error	422242.55	40	10556.06	

Table 9

Individual Comparisons for Propranolol Dose-Sex Interaction Upon Walking Scores

Comparison	df	t
Male		
Saline vs 5mg/kg	40	3.02**
Saline vs 10mg/kg	40	2.98**
Saline vs 20mg/kg	40	2.45*
Female		
Saline vs 10mg/kg	40	1.31
Saline vs 20mg/kg	40	3.01**
5mg/kg vs 20mg/kg	40	1.56

Table 10

Individual Comparisons for Propranolol Dose-Sex Interaction Upon Rearing Scores

Comparison	df	t
Male		
Saline vs 20mg/kg	40	1.29
Female		
Saline vs 5mg/kg	40	0.52
Saline vs 20mg/kg	40	2.72**
5mg/kg vs 20mg/kg	40	2.28*
10mg/kg vs 20mg/kg	40	2.69*

Table 11

Individual Comparisons for Propranolol Dose-Sex Interaction Upon Corner Scores

Comparison	df	t
Male		
Saline vs 5mg/kg	40	1.41
Saline vs 10mg/kg	40	1.32
Saline vs 20mg/kg	40	2.41*
10mg/kg vs 20mg.kg	40	1.17
Female		
Saline vs 20mg/kg	40	1.73
5mg/kg vs 20mg/kg	40	2.52*
10mg/kg vs 20mg/kg	40	1.69

APPENDIX B

Summaries of Statistical Analyses for Experiment Two

Note: * $p<.05$; ** $p<.01$

The procedure for selection of t-test comparisons followed that detailed in Appendix A.

Table 12

ANOVA Summary Table: Walking Measure

Source of Variation	SS	df	MS	F
A (sex)	36.21	1	36.21	0.57
B (dose)	198.79	3	66.26	1.04
AB	74.53	3	24.84	0.39
error	2547.36	40	63.68	

Table 13

ANOVA Summary Table: Rearing Measure

Source of Variation	SS	df	MS	F
A (sex)	481.25	1	481.25	5.99*
B (dose)	1051.73	3	350.58	4.36**
AB	167.49	3	55.83	0.69
error	3213.60	40	80.34	

Table 14

ANOVA Summary Table: Grooming Measure

Source of Variation	SS	df	MS	F
A (sex)	5.68	1	5.68	0.10
B (dose)	54.64	3	18.21	0.32
AB	187.43	3	62.48	1.10
error	2269.96	40	56.75	

Table 15

ANOVA Summary Table: Transitions Measure

Source of Variation	SS	df	MS	F
A (sex)	656.20	1	656.20	3.94
B (dose)	947.40	3	315.80	1.89
AB	1443.24	3	481.08	2.88*
error	6668.51	40	166.71	

Table 16

ANOVA Summary Table: Corners Measure

Source of Variation	SS	df	MS	F
A (sex)	42.61	1	42.61	0.13
B (dose)	2354.83	3	784.94	2.38
AB	1341.65	3	447.22	1.35
error	13211.42	40	330.29	

Table 17

ANOVA Summary Table: Centres Measure

Source of Variation	SS	df	MS	F
A (sex)	13.81	1	13.81	0.32
B (dose)	46.84	3	15.61	0.36
AB	63.78	3	21.26	0.49
error	1751.59	40	43.79	

Table 18

ANOVA Summary Table: Half Body Emergence Measure

Source of Variation	SS	df	MS	F
A (sex)	38335.97	1	38335.97	4.75*
B (dose)	73626.66	3	24542.22	3.04*
AB	43280.57	3	14426.86	1.79
error	323050.72	40	8076.27	

Table 19

ANOVA Summary Table: Total Body Emergence Measure

Source of Variation	SS	df	MS	F
A (sex)	56093.46	1	56093.46	4.70*
B (dose)	170656.33	3	56885.44	4.78**
AB	23928.50	3	7976.17	0.67
error	476172.14	40	11904.30	

Table 20

Individual Comparisons for Oxprenolol Dose Main Effect on Rearing Scores

Comparison	df	t
Saline vs 5mg/kg	40	2.63*
Saline vs 10mg/kg	40	2.67*
Saline vs 20mg/kg	40	3.54**
5mg/kg vs 20mg/kg	40	0.90

Table 21

Individual Comparisons for Oxprenolol Dose-Sex Interaction Upon Transition Scores

Comparison	df	t
Male		
Saline vs 10mg/kg	40	1.15
Saline vs 20mg/kg	40	2.35*
5mg/kg vs 20mg/kg	40	2.11*
10mg/kg vs 20mg/kg	40	1.26
Female		
5mg/kg vs 10mg/kg	40	1.74
10mg/kg vs 20mg/kg	40	2.60*

Table 22

Individual Comparisons for Oxprenolol Dose Main Effect on Half Body Emergence Scores

Comparison	df	t
Saline vs 5mg/kg	40	1.28
Saline vs 20mg/kg	40	1.61
5mg/kg vs 20mg/kg	40	2.89**
10mg/kg vs 20mg/kg	40	2.48*

Table 23

Individual Comparisons for Oxprenolol Dose Main Effect Total Body Emergence Scores

Comparison	df	t
Saline vs 5mg/kg	40	1.02
Saline vs 10mg/kg	40	0.76
Saline vs 20mg/kg	40	2.87**
5mg/kg vs 10mg/kg	40	1.78
5mg/kg vs 20mg/kg	40	3.89**
10mg/kg vs 20mg/kg	40	2.11*

APPENDIX C

Summaries of Statistical Analyses for Experiment Three

Note: * $p<.05$; ** $p<.01$

The procedure for selection of t-test comparisons followed that detailed in Appendix A.

Table 24

ANOVA Summary Table: Walking Measure

Source of Variation	SS	df	MS	F
A (sex)	351.19	1	351.19	8.75**
B (dose)	257.39	3	85.80	2.14
AB	220.46	3	73.49	1.83
error	1604.90	40	40.12	

Table 25

ANOVA Summary Table: Rearing Measure

Source of Variation	SS	df	MS	F
A (sex)	749.94	1	749.94	5.01*
B (dose)	2324.80	3	774.94	5.17**
AB	29.24	3	9.75	0.07
error	5986.79	40	149.67	

Table 26

ANOVA Summary Table: Grooming Measure

Source of Variation	SS	df	MS	F
A (sex)	3.12	1	3.12	0.03
B (dose)	175.68	3	58.56	0.53
AB	186.04	3	62.01	0.56
error	4442.87	40	111.07	

Table 27

ANOVA Summary Table: Transitions Measure

Source of Variation	SS	df	MS	F
A (sex)	7610.51	1	7610.51	22.5**
B (dose)	1912.91	3	637.64	1.89
AB	1332.23	3	444.08	1.31
error	13526.67	40	338.17	

Table 28

ANOVA Summary Table: Corners Measure

Source of Variation	SS	df	MS	F
A (sex)	275.49	1	275.49	0.81
B (dose)	1601.76	3	533.92	1.57
AB	223.02	3	74.34	0.22
error	13567.17	40	339.18	

Table 29

ANOVA Summary Table: Centres Measure

Source of Variation	SS	df	MS	F
A (sex)	0.45	1	0.45	0.88
B (dose)	344.39	3	114.80	2.24
AB	14.15	3	4.72	0.09
error	2051.93	40	51.30	

Table 30

ANOVA Summary Table: Half Body Emergence Measure

Source of Variation	SS	df	MS	F
A (sex)	48034.45	1	48034.45	6.49*
B (dose)	58486.89	3	19495.63	2.64
AB	77012.99	3	25671.00	3.47*
error	295937.28	40	7398.43	

Table 31

ANOVA Summary Table: Total Body Emergence Measure

Source of Variation	SS	df	MS	F
A (sex)	39140.67	1	39140.67	2.92
B (dose)	77307.14	3	25769.05	1.92
AB	83120.55	3	27706.85	2.07
error	535496.42	40	13387.41	

Table 32

Individual Comparisons for Atenolol Dose Main Effect on Rearing Scores

Comparisons	df	t
Saline vs 5mg/kg	40	2.35*
Saline vs 10mg/kg	40	2.24*
Saline vs 20mg/kg	40	1.01
5mg/kg vs 20mg/kg	40	3.37**
10mg/kg vs 20mg/kg	40	3.25**

Table 33

Individual Comparisons for Atenolol Dose-Sex Interaction Effect on Half Body Emergence Scores

Comparison	df	t
Male		
Saline vs 5mg/kg	40	2.33*
Saline vs 10mg/kg	40	0.32
Saline vs 20mg/kg	40	1.33
5mg/kg vs 10mg/kg	40	2.63*
5mg/kg vs 20mg/kg	40	3.47**
Female		
5mg/kg vs 10mg/kg	40	0.67